CLAIMS

1. A method of producing a modification in a gene of interest contained in a cell, comprising:

a) providing:

i) a plurality of target cells capable of being cultured;

ii) an agent capable of producing at least one modification in said gene of interest in said target cell;

b) treating said target cells with said agent under conditions such that a mixture of cells is produced, said mixture of cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest; and

c) isolating said cells having a modified gene of interest.

2. The method of Claim 1, further comprising step d) comparing the nucleotide sequence of said gene of interest in said cells having a modified gene of interest with the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest.

3. The method of Claim 2, further comprising e) manipulating said cells having a modified gene of interest to generate an organism comprising said modification in said gene of interest.

4. The method of Claim 2, further comprising prior to step d) amplifying said modified gene of interest to produce an amplified modified gene of interest.

5. The method of Claim 4, further comprising prior to step d) sequencing said amplified modified gene of interest.

6. The method of Claim 1, wherein said modification is selected from the group consisting of mutation, mismatch, and strand break.

7. The method of Claim 6, wherein said mutation is selected from the group consisting of deletion, insertion and substitution.

8. The method of Claim 6, wherein said strand break is selected from the group consisting of single-strand break and double-strand break.

9. The method of Claim 1, wherein said target cell is derived from an organism selected from the group consisting of non-human animal, plant, protist, fungus, bacterium, and virus.

10. The method of Claim 9, wherein said non-human animal is a mammal.

11. The method of Claim 10, wherein said mammal is a mouse.

12. The method of Claim 9, wherein said non-human animal is zebrafish.

13. The method of Claim 1, wherein said target cell is an embryonic stem cell.

14. The method of Claim 1, wherein said agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, $^{3}H_2O$, urethane, ultraviolet light, X-ray radiation, and gamma-radiation.

15. A method of producing an allelic series of modification in a gene of interest contained in a cell, comprising:

a) providing:

i) a plurality of target cells capable of being cultured;

ii) an agent capable of producing at least one modification in said gene of interest in said target cell;

b) treating said target cells with said agent under conditions such that a mixture of cells is produced, said mixture of cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest; and

c) isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of modification in said gene of interest.

16. The method of Claim 15, further comprising step d) comparing the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest with the nucleotide sequence of said gene of interest in cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest.

17. The method of Claim 16, further comprising e) manipulating cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

18. The method of Claim 16, further comprising prior to step d) amplifying said gene of interest selected from the group consisting of said gene of interest having said first modification and said gene of interest having said second modification to produce amplified

modified gene of interest selected from the group consisting of amplified gene of interest having said first modification and amplified gene of interest having said second modification.

19. The method of Claim 18, further comprising prior to step d) sequencing said amplified modified gene of interest.

20. The method of Claim 15, wherein said first modification and said second modification are selected from the group consisting of mutation, mismatch, and strand break.

21. The method of Claim 20, wherein said mutation is selected from the group consisting of deletion, insertion and substitution.

22. The method of Claim 20, wherein said strand break is selected from the group consisting of single-strand break and double-strand break.

23. The method of Claim 15, wherein said target cell is derived from an organism selected from the group consisting of non-human animal, plant, protist, fungus, bacterium, and virus.

24. The method of Claim 23, wherein said non-human animal is a mammal.

25. The method of Claim 24, wherein said mammal is a mouse.

26. The method of Claim 23, wherein said non-human animal is zebrafish.

27. The method of Claim 15, wherein said target cell is an embryonic stem cell.

28. The method of Claim 15, wherein said agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene

melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, $^{3}H_2O$, urethane, ultraviolet light, X-ray radiation, and gamma-radiation.